Genetic Variation in the River Sturgeon Scaphirhynchus (Acipenseridae) as Inferred from Partial mtDNA Sequences of Cytochrome *b*

FINAL REPORT

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Abstract

This report describes the amount of genetic variation that was observed within and between species of the river sturgeon of the genus *Scaphirhynchus* as determined from comparisons of a 270 base pair segment of the mitochondrial cytochrome *b* gene. The purpose of these comparisons was to determine if this gene region would be useful in the forensic identification of caviar from shovelnose sturgeon (*S. platorhynchus*) to the exclusion of pallid sturgeon (*Scaphirhynchus albus*) and Alabama sturgeon (*S. suttkusi*). Comparisons among 17 pallid sturgeon, 79 shovelnose sturgeon and 3 Alabama sturgeon revealed a total of three unique sequences (Types A, B & C) defined by three nucleotide substitutions for a maximum divergence of 1.1%. All three sequence types co-occurred in two shovelnose populations, whereas two sequence types were found together in pallid and shovelnose populations. All three Alabama sturgeon exhibited sequence type A. Northern populations of pallid and shovelnose sturgeon exhibited sequence type A exclusively, while types B and C were observed (along with type A) in both species at more southern localities.

Although intraspecies variation was evident in pallid and shovelnose sturgeon, it was less than 1.1%, and no unique nucleotide substitutions were observed in either pallid, shovelnose or Alabama sturgeon. Similar characterizations of 122 individuals representing 13 different species in the related sturgeon genus *Acipenser* revealed an average interspecies sequence divergence of 5.8% and a maximum intraspecies sequence divergence of 1.5%. In addition, three species groups in *Scaphirhynchus* and *Acipenser* that taxonomists have in the past regarded as conspecific, but now consider to be distinct species (i.e., *Acipenser persicus – A. gueldenstaedtii*; *A. medirostris – A. mikadoi*; and

Scaphirhynchus albus – S. platorhynchus – S. suttkusi) were indistinguishable.

Introduction

The sturgeon/paddlefish (order Acipenseriformes) are primitive bony fish that evolved some 200 million years ago (Bemis et al 1997). The order is comprised of twenty-seven species in all, nine of which occur in North America. The North American sturgeon/paddlefish include three distinct genera: *Scaphirhynchus*, *Acipenser* and *Polyodon*. Both sturgeon and paddlefish have been severely reduced throughout much of their range in North America due to intensive commercial fishing for meat and caviar, and habitat perturbation in the last century (Birstein 1993). Consequently, two species: shortnose sturgeon (*A. brevirostrum*) and pallid sturgeon (*S. albus*), have been listed as endangered under the US Endangered Species Act (ESA) and a third, the Alabama sturgeon (*S. suttkusi*), is currently being considered for listing.

Taxonomists currently recognize three different species in the genus *Scaphirhynchus*: pallid sturgeon (*S. albus*), shovelnose sturgeon (*S. platorhynchus*) and Alabama sturgeon (*S. suttkusi*) (Bailey & Cross 1954, Mayden & Kuhajda 1996). Although rarely encountered, pallid sturgeon still range throughout the Missouri River and south from its confluence with the Mississippi River into Louisiana. In contrast, shovelnose sturgeon occur throughout the greater Mississippi River drainage in sufficient numbers to support sport and commercial fisheries (Keenlyne 1997). However, the plight of the Alabama sturgeon would appear most extreme as, throughout its historic range in the Mobile River drainage of Alabama and Mississippi, only thirteen live captures have been documented since 1985. Chermock (1955) originally described the Alabama

sturgeon as *S. platorhynchus* but recently Williams & Clemmer (1991) and Mayden & Kuhajda (1996) have used morphometrics to elevate it to distinct species status (*S. suttkusi*).

A responsibility of the National Fish and Wildlife Forensic Laboratory is to facilitate sturgeon conservation by providing analytical support for the enforcement of the ESA and the Convention on International Trade in Endangered Species (CITES) concerning foreign and domestic trade in sturgeon products. As commercial fisheries presently exist for the shovelnose sturgeon, and pallid and Alabama sturgeon could well be exterminated by illegal poaching associated with these fisheries, it is critically important to know which species are being traded and the proportion of the trade they comprise. To that end, we sought to develop a DNA procedure for the forensic identification of the caviar and meat of the river sturgeon *Scaphirhynchus.*

The mitochondrial cytochrome *b* gene has been used extensively as a genetic marker for vertebrate species distinction and, as a result, its evolutionary dynamics are well understood (Irwin et al 1991). The 5' end of cytochrome *b* has been shown to exhibit significant interspecific variation in sturgeon (Schill & Walker 1994, Birstein et al 1997) and other fish species (e.g., shark: Martin et al 1992; rockfish: Rocha-Olivares et al 1999). Consequently, we investigated this portion of cytochrome *b* as a means to positively identify individual sturgeon species, including pallid, shovelnose and Alabama sturgeon. We characterized sequence variation in a 270 bp portion of the cytochrome *b* gene (amino acid positions 36-125; nucleotide positions 108-378; Brown et al 1989) in 247 individual sturgeon species type standards. These included pallid, shovelnose and Alabama sturgeon as well as sixteen other sturgeon and paddlefish species.

Materials & Methods

Sampling design

The 245 sturgeon and paddlefish standards used for the development of type sequences are listed in Table 1 along with the number of individuals characterized, and the source of each sample. Assessments of intraspecific variation in the targeted region of cytochrome *b* included comparisons of 17 samples from three populations of *Scaphirhynchus albus*, 79 samples from seven populations of *S. platorhynchus* and 3 samples from one population of *S. suttkusi* (Table 2). Within *Acipenser*, an additional 24 samples from two populations of *A. transmontanus*; 10 samples from two populations of *A. medirostris*; and 7 samples of *Acipenser oxyrinchus oxyrinchus*, and 11 samples of *A. o. desotoi* were compared (Table 2). The type sequences from multiple accessions (range = 2 to 23, Table 1) of the thirteen remaining sturgeon species were also examined for intraspecies variation. Total cellular DNA was prepared from muscle tissue, whole blood or single eggs after the method of Boom et al (1990).

Cytochrome b Sequences.

Sequences from a 314 base pair segment of the cytochrome *b* gene were obtained by polymerase chain reaction (PCR) amplification with primers L14851-13 & H14997 (Table 3). The PCR amplifications were performed in 50 µL reaction volumes containing about 100 ng of template DNA (1-10 µL extract volume), 20 mM Tris (pH 8.4), 50 mM KCl, 2.0 mM $MgCl_2$, 200 µM concentration for each nucleotide, 1 µM concentration for each primer, Bovine Serum Albumin (160 µg/mL) and 2.5 units of Taq DNA polymerase (Life Technologies). Amplification was accomplished in a programmable heating block (Model 9600; Perkin Elmer) and the amplification profile

included 30 cycles of denaturing for 1 minute at 95°C, primer annealing for 45 seconds at 55°C, and extension for 45 seconds at 72°C. Positive and negative amplification controls were included in each run.

Amplification products were purified of unincorporated nucleotides and primers in dyalizing spin columns (Microcon-30, Millipore), brought to a final volume of 20 μL in sterile water and sequenced with the ABI PRISM BigDye Terminator Cycle Sequencing protocol (Perkin Elmer, 1998). Each sequencing run included the plasmid pGEM-3Zf(+) (Promega) as a positive sequencing control. Sequences were characterized on an automated DNA sequence analyzer (Model 377, Perkin Elmer), and the region of the cytochrome *b* gene compared was 270 base pairs in length (amino acid positions 36-125; nucleotide sites 108-378).

Data analysis

The cytochrome *b* sequences of the type samples were aligned with the DNA Sequence Editor SeqEd (ver. 1.00A, Applied Biosystems, 1990). The genetic distance algorithm DNADIST of PHYLIP (Phylogeny Inference Package version 3.4; Felsenstein 1993), was used to calculate total uncorrected sequence divergence among the nineteen sturgeon and paddlefish type sequences in Table 1.

Results

Interspecies variation within Acipenser

Alignments of 270 bp of the 5' end of the cytochrome *b* gene identified a total of 76 variable sites that defined 24 unique sequences in 246 type standards of 19 sturgeon/paddlefish species (Table 4). Sequence divergence between the 13 species of

Acipenser ranged from 0-10.4% (Table 5). Both of the *A. persicus* standards and eight of the ten *A. gueldenstaedtii* standards were identical (Table 4). All of the *A. medirostris* and *A. mikadoi* sequences were identical (Table 4). The *A. transmontanus* and *A. schrenckii* type sequences were distinguished at just one site (0.4% sequence divergence, Tables 4 & 5).

Sequence variation within Scaphirhynchus

Comparisons among 17 pallid sturgeon (*Scaphirhynchus albus*), 79 shovelnose sturgeon (*S. platorhynchus*) and 3 Alabama sturgeon (*S. suttkusi*) revealed a total of three unique sequences defined by three nucleotide substitutions for a maximum divergence of 1.1% (Tables 4 & 6). All three sequence types were present together in two populations of *S. platorhynchus* whereas two sequence types were found together in two populations of *S. albus*, and one population of *S. platorhynchus*. The three Alabama sturgeon sequences were identical. Northern populations of pallid and shovelnose sturgeon exhibited sequence type A (SsuH10432, Table 4) exclusively, while types B and C (SplF41211 and SalB40604 respectively, Table 4) appear (along with type A) in both species at more southern localities (Table 6). Divergent types differed from the most common type (SsuH10432 was exhibited by 86% of shovelnose sturgeon) by 1-2 substitutions (0.4-0.8% sequence divergence, Tables 4 & 6). Intraspecies variation was evident in pallid and shovelnose populations, but it was less than 1.1%. Interspecies variation was also equal to 1.1%, but no unique substitutions were observed in either species.

Intraspecies variation within Acipenser

For purposes of comparison, no intraspecies variation was detected in the target region of cytochrome *b* for *A. transmontanus* - all 24 type sequences were identical. The 10 *A. medirostris* standards were also identical; as were all 18 *A. oxyrinchus oxyrinchus* and *A. o. desotoi* standards (Table 2). In addition, *A. ruthenus* and *A. stellatus* exhibited 3 unique sequences respectively (0.4-0.8% sequence divergence), and *A. gueldenstaedtii*, and *A. baerii* each exhibited 2 unique sequences (0.4% sequence divergence). However, no intraspecies variation was detected in the sequences from multiple accessions of *A. brevirostrum, A. fulvescens, A. mikadoi, A. nudiventris, A. persicus,* and *A. schrenckii* (Table 4).

Discussion

Interspecific variation

As expected for ancient species such as sturgeon and paddlefish, we consistently observed significant interspecies sequence divergence. Sequence divergence between non-identical species of *Acipenser* ranged from 0.4-10.4%. The average interspecies sequence divergence was 5.8%. However, both of the *A. persicus* standards and eight of the ten *A. gueldenstaedtii* standards were identical, all of the *A. medirostris* and *A. mikadoi* sequences were identical, and the *A. transmontanus* and *A. schrenckii* type sequences were distinguished at just one site (0.4% sequence divergence).

Intraspecific variation

Consistant with characterizations of other fish species (e.g., tuna: 1.0-2.0%, Bartlett & Davidson 1991; cod: 0-1.1%, Carr & Marshall 1992; stickleback: 0.4-3.1%,

Orti et al 1994; rainbow fish: 0.5-3.6%, Zhu et al 1994) we observed low levels of intraspecific cytochrome *b* diversity in *Scaphirhynchus*. The three Alabama sturgeon were identical, and both within and between species sequence divergence in pallid and shovelnose sturgeon, was less than 1.1%. The maximum intraspecies sequence divergence observed in this study was 1.5% among the *A. gueldenstaedtii* and *A. stellatus* species groups, followed by 1.1% sequence divergence among the *A. baerii* type sequences. Moreover, tests designed to estimate geographic variation in populations of *A. transmontanus, A. medirostris* and the subspecies *A. oxyrinchus oxyrinchus* and *A. o. desotoi* did not detect any variation at all. In what might be considered a contradictory result, representatives of the three species groups *A. persicus – A. gueldenstaedtii*; *A. medirostris – A. mikadoi*; and *Scaphirhynchus albus – S. platorhynchus – S. suttkusi* were also identical. It should be noted, however, that taxonomists have regarded these species groups as conspecific in the past (Bailey & Cross 1954, Birstein & Bemis 1997).

Conclusion

The forensic identification of caviar from shovelnose sturgeon (*S. platorhynchus*) to the exclusion of pallid sturgeon (*Scaphirhynchus albus*) and Alabama sturgeon (*S. suttkusi*) remains elusive. This is not for want of effort, however. Two nuclear marker studies of pallid and shovelnose sturgeon using allozymes (Phelps & Allendorf 1983) and annonymous DNA markers (Genetic Analyses, Inc. 1994) failed to identify species specific variants useful for the forensic identification of caviar. Two other previous studies compared DNA sequences of cytochrome *b*. Schill and Walker (1994) compared single representatives of pallid, shovelnose and Alabama sturgeon; while Heath &

Mayden (1996) compared 5 pallid, 4 shovelnose and 3 Alabama sturgeon. Neither of these studies identified species specific variants useful for forensic identification either. Finally, in the most comprehensive effort to date, Campton et al (1999) compared partial DNA sequences of the mitochondrial control region from 29 pallid, 37 shovelnose and 3 Alabama sturgeon. Although a single fixed unique substitution was observed in Alabama sturgeon, no such distinction was found between pallid and shovelnose sturgeon. The present study, also reasonably comprehensive, compared partial DNA sequences of cytochrome *b* from 19 pallid, 79 shovelnose and 3 Alabama sturgeon. However, although the suggestion of a geographic pattern to the distribution of sequence types was noted, we also failed to identify any substitutions unique to either pallid, shovelnose or Alabama sturgeon.

These data for *Scaphirhynchus* and the intraspecies/interspecies comparisons we report for *Acipenser* suggest that the river sturgeon, like many other forensically relevant species, are only very recently diverged. In these situations the fixed allelic differences that diagnostic species tests require are rare. We are currently searching for new diagnostic markers in both the mitochondrial and nuclear genomes of river sturgeon. We are hopeful as our efforts in this endeavor continue.

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TABLE 1. List of the species, number of specimens, collection locality and contributor of the sturgeon/paddlefish type standards used in this study.

Species	Geographical location	Contributor
Family: Acipenseridae		
Subfamily: Acipenserinae		
Genus *Acipenser*		
A. baerii (n=23)[1]	- Welaka National Fish Hatchery, FL	- Allan Brown, USFWS
	- Russian Federation	- Lutz Debus, Rostock University
A. brevirostrum (n=2)	- Connecticut River, MA	- Micah Kieffer, S.O. Conte Center
A. fulvescens (n=6)	- Lake Winnebago, WI	- Ron Bruck, WI DNR
	- Mississippi River, IA	- Maury Anderson, IA DNR
	- Charleston, SC	- Gloria Seaborn, NMFS
	- Chippewa River, WI	- Ann Runstrom, USFWS
A. gueldenstaedtii (n=10)	- Russian Federation	- Allan Brown, UWFWS
	- Volga River, Russian Federation	- Lutz Debus, Rostock University
	- Germany	- Caroline Raymakers, Traffic Europe
	- Hungary	
A. medirostris (n=11)	- Klamath River, CA	- Bruce Oppenheim, CA F&W
		- Dave Hillemeier, Yurok Tribe
	- Lower Columbia River, OR	- Kevleen Melcher, OR Dept. F&W
A. mikadoi (n=2)	- Russian Federation	- Lutz Debus, Rostock University
A. nudiventris (n=2)	- Russian Federation	- Lutz Debus, Rostock University
A. oxyrinchus oxyrinchus (n=7)	- Solomon's Island, MD	- Troy Gunderson, Chesapeake Biological
A. oxyrinchus desotoi (n=11)	- Washington County, FL	Lab
	- Santa Rosa, FL	- Frank Parauka, USFWS
A. persicus (n=2)	- Volga River, Russian Federation	
A. ruthenus (n=9)	- Germany	- Lutz Debus, Rostock University
	- Hungary	- Caroline Raymakers, Traffic Europe
	- Russian Federation	
A. schrenckii (n=6)	- Heilongjiang River, Fuyuan, China	- Lutz Debus, Rostock University
A. stellatus (n=8)	- Volga River, Russian Federation	- Caroline Raymakers, TrafficEurope
	- Hungary	- Lutz Debus, Rostock University
A. transmontanus (n=22)	- Lower Sacramento River, CA	- Caroline Raymakers, Traffic Europe
	- Lower Columbia River, OR	- Joel Van Eenennaam, UC-Davis
		- Kevleen Melcher, OR Dept. F&W
Genus *Huso*		
H. dauricus (n=11)	- Heilongjiang River, Fuyuan, China	- Caroline Raymakers, TrafficEurope
H. huso (n=7)	- Volga River, Russian Federation	- Lutz Debus, Rostock University
	- Moscow, Russian Federation	
Subfamily: Scaphirhynchinae		
Genus *Scaphirhynchus*		
S. albus (n=17)	- Missouri River, ND	- Kent Keenlyne
	- Atchafalaya River, LA	
	- Mississippi River, MO	- Trent Lane & Michael Petersen, MO Dept. of Conservation
S. platorhynchus (n=79)	- Yellowstone River, MT	- Phil Stewart, MT FWP
	- Mississippi River, MO	- Trent Lane & Michael Petersen, MO Dept. of Conservation
	- Rock Island, MD	- Ken Phillips, USFWS
	- Vicksburg, MS	- Jack Kilgore, US Army COE
	- Cedar County, NE	- Fries & Whitmore
	- Missouri River, SD	- Richard Ruelle, USFWS
	- Atchafalaya River, LA	- Kent Keenlyne
*S. suttkus*i (n=3)	- Alabama River, AL	- Mike Howell, Samford University
		- Marion Fish Hatchery
Family: Polyodontidae		
Polyodon spathula (n=4)	- Dawson County, MT	- Phil Stewart, MT FWP
	- Mermentau River, LA	- Philip Siragusa, USFWS/LE
	- Atchafalaya Basin, LA	
	- Chippewa River, WI	- Ann Runstrom, USFWS

[1]n=Number of specimens analyzed.

TABLE 2. List of species, collection localities and contributors of the sturgeon type standards used in intra-species variation assessments.

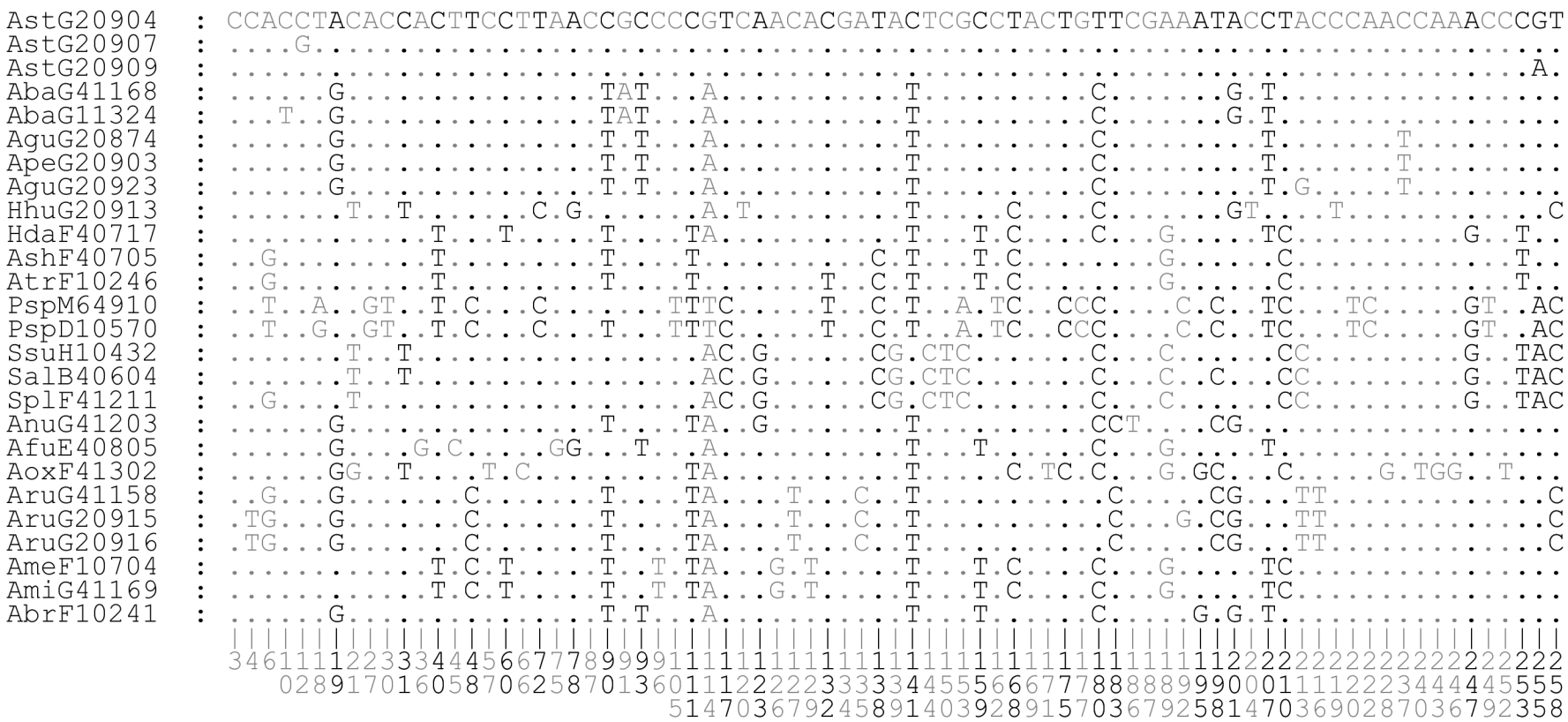
Species	Geographical Location	Samples Analyzed	Name of Contributor
Family: Acipenseridae			
Subfamily: Acipenserinae			
Genus *Acipenser*			
A. medirostris	- Klamath River, CA	6	- Bruce Oppenheim, CA F&W
			- Dave Hillemeier, Yurok Tribe
	- Lower Columbia River, OR	4	- Kevleen Melcher, OR Dept. F&W
A. oxyrinchus oxyrinchus	- Chesapeake Bay, MD	7	- Troy Gunderson, Chesapeake Biological Lab
A. oxyrinchus desotoi	- Washington County, FL	6	- Frank Parauka, USFWS
	- Santa Rosa, FL	5	
A. transmontanus	- Lower Sacramento River	7	- Joel Van Eenennaam, UC-Davis
	- Lower Columbia River	17	- Kevleen Melcher, OR Dept. F&W
Subfamily: Scaphirhynchinae			
Genus *Scaphirhynchus*			
S. albus	- Missouri River, ND	6	- Kent Keenlyne
	- Atchafalaya River, LA	5	
	- Mississippi River, MO	6	- Trent Lane & Michael Petersen, MO Dept. of Conservation
S. platorhynchus	- Yellowstone River, MT	6	- Phil Stewart, MT FWP
	- Mississippi River, MO	40	- Trent Lane & Michael Petersen, MO Dept. of Conservation
	- Rock Island, MD	6	- Ken Phillips, USFWS
	- Vicksburg, MS	11	- Jack Kilgore, US Army COE
	- Cedar County, NE	8	- Fries & Whitmore
	- Missouri River, SD	7	- Richard Ruelle, USFWS
	- Atchafalaya River, LA	1	- Kent Keenlyne
S. suttkusi	- Alabama River, AL	3	- Mike Howell, Samford University
			- Marion Fish Hatchery

Table 3. List of the primers used in this study for amplification and sequencing of sturgeon cytochrome *b*.

Name	Sequence	Usage	Ref
L14769	5'-TGTAAAACGACGGCCAGTGACCAACATCCGAAAAACAC-3'	P,C	this study
H15149	5'-CTCAGAATGATATTTGTCCTCA-3'	P	Kocher *et al.* 1989
L14851-13	5'-TGTAAAACGACGGCCAGTGTGTGATGAAATTTTGGCTC-3'	P,C	this study
H15125	5'-AGTACATATCCTACGAAGGC-3'	P	this study
L15021-13	5'-TGTAAAACGACGGCCAGTTTCATGCAAACGGGGCCTC-3'	P,C	this study
H14996	5'-GAAAGAGGCCCCGTTTGC-3'	P	this study

Names identify the DNA strand (H/L) and the position of the 3' end of the primer according to the numbering system for the human mtDNA sequence (Anderson *et al.* 1981). Primers were used in primary amplifications (P) and in cycle sequencing amplifications (C). Underlined sequences are complementary to the -21M13 sequencing primer.

TABLE 4. Nucleotide composition at variable sites in cytochrome b target region for 26 standard type sequences



Abbreviations used in the labels at the right hand column to identify the species source of each type sequence: Aba (Acipenser baerii), Abr (Acipenser brevirostrum), Afu (Acipenser fulvescens), Agu (Acipenser gueldenstaedtii), Ame (Acipenser medirostris), Anu (Acipenser nudiventris),Aox (Acipenser oxyrinchus), Aru (Acipenser ruthenus), Asc (Acipenser schrenckii), Ast (Acipenser stellatus), Atr (Acipenser transmontanus), Hda (Huso dauricus), Hhu (Huso huso), Psp (Polyodon spathula), Sal (Scaphirhynchus albus), Spl (Scaphirhynchus platorhynchus), Ssu (Scaphirhynchus suttkusi). Columns in bold type denote phylogenetically informative sites.

Table 5. Sequence divergence (uncorrected) found in pair-wise comparisons of pallid, sturgeon along with the sixteen other species studied. Values are expressed in percentages. See Table 4 for the sturgeon/paddlefish species represented by each three-letter label.

	Aba	Abr	Afu	Agu	Ame	Ami	Anu	Aox	Ape	Aru	Asc	Ast	Atr	Hda	Hhu	Sal	Psp
Aba		3.7-4.1	3.3-3.7	1.5-1.9	5.6-5.9	5.6-5.9	2.9-3.3	8.2-8.5	1.5	5.2-6.3	5.9-6.3	3.3-4.4	6.3-6.7	4.8-5.2	5.6-6.3	8.9	13.0
Abr			5.6	4.1-4.4	7.4	7.4	5.9	10.4	4.1	8.9	7.4	5.2-5.9	7.8	6.3	7.8-8.1	10.7	15.2
Afu				3.0-3.3	5.9	5.9	5.6	8.5	3	3.0	6.3	4.4-5.2	6.7	5.2	6.7-7.0	8.5	14.4
Agu					4.8-5.2	4.8-5.2	3.3-3.7	7.8-8.1	0-0.4	5.6-6.3	5.6-5.9	3.3-4.4	5.9-6.3	4.1-4.4	5.6-6.3	7.8	12.6
Ame						0	6.3	8.5	4.8	8.9	4.1	6.3-7.0	4.4	2.2	8.1-8.5	10.4	12.2
Ami							6.3	8.5	4.8	8.9	4.1	6.3-7.0	4.4	2.2	8.1-8.5	10.4	12.2
Anu								7.4	3.3	4.4	5.9	4.1-4.8	6.3	5.6	6.3-6.7	8.5	13.3
Aox									7.8	10.4	8.1	7.8-8.5	8.5	7.8	8.9-9.3	10.7	15.2
Ape										5.6-6.3	5.6	3.0-3.7	5.9	4.1	5.6-5.9	7.4	12.2
Aru											6.7-7.4	5.6-6.7	7.0-7.8	8.5	8.9	11.1	14.1
Asc												4.1-5.2	0.4	2.6	7.8-8.1	8.1	11.9
Ast													4.4-5.6	5.2-5.9	5.2-5.9	7.0	12.6
Atr														3.0	8.1-8.5	8.5	11.5
Hda															7.4-7.8	7.8	11.5
Hhu																8.5	13.7
Sal																	13.3
Psp																	

Table 6. Occurrence of cytochrome *b* sequence types in pallid, shovelnose and Alabama sturgeon at different localities.

Population	Pallid			Shovelnose			Alabama		
	A	B	C	A	B	C	A	B	C
Missouri R., ND	6								
Yellowstone R., MT				6					
Missouri R., SD				7					
Vicksburg, MS				7	3	1			
Cedar Co., NE				7	1				
Mississippi R., MO	1		5	34	3	3			
Rock Is., MD				6					
Atchafalaya R. LA		1	4	1					
Alabama R., AL							3		
Totals	7	1	9	68	7	4	3	0	0

Type A=SsuH10432, type B=SplF41211and type C=SalB40604 in Table 6.
Sequence divergence between types A & B = 0.7%; between types A & C = 0.4% and between types B & C = 1.1%.